REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

Initially, Applicant acknowledges with thanks the Examiner's indication in the Office Action that all former grounds of rejection have been overcome except for the rejection of the claims under 35 USC 103 over the combination of Stavrianopoulos et al. and Matkovich et al.

A feature of the previous claims is that the apparatus includes "an array of oligonucleotides with predetermined sequences". As noted on page 7 of May 29, 2007, this phrase was accepted as definite during examination of parent patents 5,700,637 and 6,054,270 and was explicitly taken by the PTO to mean that the sequences of the immobilised oligonucleotides are known.

In section 16 of the Office Action, the Examiner argues that the term "predetermined" in the previous claims did not imply that the sequences were "known", despite the history of inventor's '637 and '270 patents. To address this issue, the term "predetermined" has been replaced by "known". This term finds basis in section 5.1 of the description, where the inventor writes that positive cells (*i.e.* those giving a signal after a hybridisation experiment) "represented known sequences". Moreover, the inventor's work in the examples, as illustrated in Table 1, clearly show that he was working with "known" sequences. Entry and consideration is respectfully submitted to be appropriate and is requested.

Thus it is clear that the claimed apparatus requires an array of oligonucleotides in which the complete sequence of each and every oligonucleotide probe on the array surface is known.

Claims 17-27 and 86-87 are rejected under 35 USC 103 as obvious from Stavrianopoulos and Matkovich. This ground of rejection is respectfully traversed.

Neither cited reference teaches or suggests the apparatus of claims 17-27 as amended. Claims 17-27 require "a support having an impermeable surface; porous material attached to the impermeable surface; and an array of oligonucleotides with known sequences attached to the porous material".

Neither cited reference teaches or suggests attaching an array of oligonucleotides with known sequences to a support according to claim 17. Stavrianopoulos describes products in the opposite orientation to the claimed invention. In Stavrianopoulos a nucleic acid of unknown sequence to be analyzed is immobilized on a support, and a probe with a known sequence is applied to the immobilized unknown sequence (a "target down" assay). None of the products disclosed by Stavrianopoulos has an immobilized oligonucleotide with a known sequence. The immobilized sequences in the Stavrianopoulos method are the analyte having an unknown sequence rather than the probe.

Regarding claims 23, 24, 86 and 87, the Applicant can find no disclosure in Stavrianopoulos of a covalent attachment of the oligonucleotides to the support according to claims 23 and 86, or a terminal attachment of the oligonucleotides to the support according to claims 24 and 87.

Column 5, line 58 to column 6, line 4 of Stavrianopoulos is referenced in the rejection. This disclosure teaches covalently attaching a chemical label to the probe. This disclosure is irrelevant to covalently attaching an array of oligonucleotides having a known sequence to a support.

Column 8, lines 11-56 of Stavrianopoulos (Example 1) is also referenced in the rejection. However this disclosure relates to immobilizing or "fixing" a negatively charged oligonucleotide to a support by coating the support with a positively charged alkylamine layer. Example 1 binds DNA to the glass by a +ve/-ve interaction. Thus the Stavrianopoulos method involves ionic attachment of the oligonucleotides to the support, not covalent attachment according to the claimed method.

Matkovich does mention covalent binding once (col. 4, last line), but only in passing and only with regards to an antibody assay. Matkovich's initial suggestion is physical bonding, and covalent chemical coupling is merely mentioned as "some manner" of attachment.

Stavrianopoulos mentions a "parallel" analysis system, where the analyte sequences are immobilized in different reaction containers e.g. different wells of a microtitre plate. These different wells on a plate are not "an impermeable surface" of a support as claimed; rather, they are separate surfaces. For example, a sample applied to

one well would not be able to hybridize to nucleic acids in another well, because the immobilized nucleic acids are on separate surfaces.

Furthermore, a skilled person would not reasonably have combined the teachings of Stavrianopoulos with Matkovich. Stavrianopoulos deals with nucleic acid assays. Matkovich on the other hand is explicitly concerned with antibody assays. See for example the "Background" section, culminating with the statement "Accordingly, there remains a need for improvements in multiwell plates to provide for *** increased antibody binding *** in a more reliable manner". In addition, the "Summary of the Invention" section refers to surfaces "capable of binding antibody", etc.

A skilled person starting with the DNA assay of Stavrianopoulos would not have looked to the teachings of Matkovich because it is from a different technical field (antibodies vs. DNA). Such combination is respectfully submitted to be hindsight. Even if a skilled person had looked at Matkovich to improve DNA assays, they would not be guided in particular to choose covalent bonding because (i) it is mentioned only in passing, with other attachments being equally likely, and (ii) Matkovich concerns antibodies and so offers no useful teaching about how to attach DNA. Thus, the rejection requires the skilled person to make an unlikely combination of documents, and then to make a specific selection that was not made by Matkovich himself. This chain of connections would not have been made by one of ordinary skill.

Stavrianopoulos had already considered attachment of DNA to porous materials, but found them less desirable. Please see the end of the third paragraph in "Summary of the Invention" section. Why would the skilled person reject Stavrianopoulos's preference, then look to an antibody-related document, and then select covalent attachment in particular, to improve upon the DNA assay of Stavrianopoulos? It is respectfully submitted that such combination of selected teachings in the art must be based upon improper picking and choosing using hindsight.

Moreover, there is no mention or suggestion in Stavrianopoulos or Matkovich of a covalent terminal attachment of nucleic acids to a support, according to claims 24 and 87.

In summary, the claims are not obvious from the cited references because:

- Stavrianopoulos does not disclose covalent attachment
- Stavrianopoulos does not disclose an array with “known” sequences

Stavrianopoulos does not disclose covalent attachment

At the top of page 5 of the Office Action, the examiner argues: "Oligonucleotides or oligonucleotide probe sequences (col. 5, line 58 to col. 6, line 4) are covalently attached to the support after the glass has been treated with a silane linker for covalent attachment (col. 8, Example) and wherein the DNA can hybridize to the plates (col. 12, example 7) which involves the binding with terminal nucleotide, as in claim 23 and 24."

On pages 7-8, in section 18, the examiner argues: "Stavrianopoulos *et al.* teaches treatment of the array with a silane linker (the amma*[sic]*-aminopropyltriethoxysilane of Example 1) for covalent attachment of DNA."

The examiner's interpretation of Stavrianopoulos is incorrect. Stavrianopoulos mentions the use of silanes, but these specific linkers result in ionic bonding of DNA rather than covalent bonding.

Example 1 in Stavrianopoulos does treat glass with γ-amino-propyl-triethoxy-silane (col. 8, lines 23*ff*), but this silane is used for ionic bonding of nucleotides. The silane becomes attached to the glass surface and its amino group is left outward-facing. Thus the surface becomes covered in amino groups, which are protonated to leave multiple positively-charged amine groups exposed on the surface. The amine is linked via the silane's propyl group down to the silicon region of the surface. This situation is confirmed at lines 32-35: "The resulting treated glass surface will now have available alkylamine thereon suitable for immobilizing or fixing any negatively charged polyelectrolytes applied thereto."

Thus the exposed amines form non-covalent bonds with random negative phosphate groups on a nucleic acid's backbone. The bonding in this situation is neither covalent nor by a terminal nucleotide, and so the examiner's interpretation of Stavrianopoulos is incorrect.

If technical evidence of the non-covalent nature of these binding chemistries is required, the Applicant refers the examiner to the first page of Dawson *et al.,* 2005 (page attached): "Noncovalent immobilization (including the use of polylysine-coated and aminopropylsilane-coated slides) is commonly used for gene expression microarrays ...".

Later in Stavrianopoulos, Example 5 uses either an amino-substituted hydrophobic polymer (column 10, line 63) or poly-lysine (column 11, line 2) to treat the surface of polystyrene. The amino groups here again serve to give a positively-charged aminated surface.

Example 6 mentions the use of 6-aminohexane (column 11, line 60) which, again, gives an positively-charged amine-covered surface. The next method described in Example 6 is confirmed at column 12, line 14, to give a "polyamine polymeric coating".

Finally, Example 7 of Stavrianopoulos merely dilutes DNA in ammonium acetate, applies it to wells and leaves the aqueous material to evaporate (column 12, lines 25-47).

Thus none of the examples in Stavrianopoulos discloses covalent attachment of nucleic acids to the support material, and *a fortiori* none discloses covalent attachment via a terminal nucleotide.

The disclosure of covalent attachment in Matkovich (column 4, last line) is merely in a list of possible attachment chemistries, and is in a document that concerns antibody assays. There is no disclosure of covalent attachment of a nucleic acid oligonucleotide, and certainly no disclosure of attachment via a terminal nucleotide thereof.

As recent confirmation of the advantages of covalent attachment in DNA arrays, we refer again to the first page of Dawson *et al.,* 2005 (page attached): "... a single terminal covalent attachment is preferred for short oligonucleotides. This terminal covalent attachment allows the entire oligonucleotide to be available for hybridization and to withstand the high temperatures and salt concentrations often required during the stringent washing conditions in subsequent steps of microarray processing." These advantages flow directly from the array synthesis methods used in the present application and were not suggested by either Stavrianopoulos or Matkovich

Thus reconsideration and allowance is respectfully requested of claims 23, 24 and 87.

Stavrianopoulos does not disclose an array with "predetermined" sequences

The immobilised sequences in Stavrianopoulos are unknown. The assay format in Stavrianopoulos uses immobilised unknown analyte and applies to these a known probe sequence in the solution phase.

In contrast, the presently-claimed arrays comprise immobilised known sequences, to which an unknown analyte is applied.

It is clear that the combined teachings of the cited references fail to suggest the claimed invention. Thus reconsideration of all claims and allowance is respectfully requested.

Respectfully submitted,

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Spotting optimization for oligo microarrays on aldehyde–glass

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Abstract

Low-density microarrays that utilize short oligos (<100 nt) for capture are highly attractive for use in diagnostic applications, yet these experiments require strict quality control and meticulous reproducibility. However, a survey of current literature indicates vast inconsistencies in the spotting and processing procedures. In this study, spotting and processing protocols were optimized for aldehyde-functionalized glass substrates. Figures of merit were developed for quantitative comparison of spot quality and reproducibility. Experimental variables examined included oligo concentration in the spotting buffer, composition of the spotting buffer, postspotting "curing" conditions, and postspotting wash conditions. Optimized conditions included the use of 3–4 μM oligo in a 3× standard saline citrate/0.05% sodium dodecyl sulfate/0.001% (3-[(3-cholamidopropyl) dimethylammonia]-1-propane sulfonate) spotting buffer, 24-h postspotting reaction at 100% relative humidity, and a four-step wash procedure. Evaluation of six types of aldehyde-functionalized glass substrates indicated that those manufactured by CEL Associates, Inc. yield the highest oligo coverage.

Keywords: Low-density microarrays; Diagnostics; Spotting solution; Aldehyde substrates; Reproducibility

Microarray technology is extremely powerful for massively parallel differential gene expression analyses [1] in which relatively long cDNA fragments (>100 nt) are immobilized on a substrate and exposed to cDNAs that derive from mRNA. These cDNAs are made from "treated" and "untreated" states that are differentially fluorophore-labeled. The ratio of the two fluorescence signals serves as a measure of changes in mRNA levels. Many experimental and technological challenges must be addressed to make the data generated from these experiments more reproducible [2–7]. As many reviews and publications have outlined [8–17], the most pressing difficulties in obtaining reliable data for these multiplex analyses lie in the areas of sequence selection [12–17] and methods of data analysis [8–11]. DNA microarrays are also increasingly attractive for diagnosis as research directed at diagnostic applications is rapidly expanding and ranges from microbiology to clinical virology [18–21]. The oligonucleotide microarrays used for diagnostic applications tend to be lower in density and employ short oligos [20–22]. Here we define "short" oligonucleotides to be those up to 100 nucleotides in length, which can be synthesized using standard phosphoramidite chemistry and/or purchased commercially.

A number of noncovalent and covalent chemistries can be used to immobilize oligos on a glass substrate [22]. Noncovalent immobilization (including the use of polylysine-coated and aminopropylsilane-coated slides) is commonly used for gene expression microarrays, whereas a single terminal covalent attachment is preferred for short oligonucleotides. This terminal covalent attachment allows the entire oligonucleotide to be available for hybridization and to withstand the high temperatures and salt concentrations often required during the stringent washing conditions in subsequent steps of microarray processing. Direct covalent coupling is most commonly achieved using amino-terminated oligos and

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